Annex H

ICCVAM/NICEATM BG1Luc4E2 ER TA – Validation Work Plan

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VALIDATION OF THE BG1LUC4E2 ER TA ASSAY FOR THE DETECTION OF ESTROGEN RECEPTOR AGONISTS AND ANTAGONISTS

STUDY DESIGN and WORK PLAN

08 November 2010

TABLE OF CONTENTS

STUDY DESIGN and WORK PLAN

Validation of the BG1LUC4E2 ER TA for the Detection of Estrogen Agonists and Antagonists

1.0 PROJECT OBJECTIVES AND GENERAL REQUIREMENTS

1.1 Project Objectives

This document specifies the procedures that participating laboratories will use to conduct the international validation study of an estrogen receptor (ER) transcriptional activation (TA) assay (BG1LUC4E2 ER TA assay) for the detection of ER agonists and antagonists. The list of 78 ICCVAM recommended substances, which possess varying degrees of ER agonist and/or antagonist activity (ICCVAM 2002; ICCVAM 2003; Federal Register, Vol. 71, No. 51, pp. 13597-13598, March 16, 2006;), will be used in this validation study to characterize the reliability and relevance of the BG1LUC4E2 ER TA assay.

1.2 General Capabilities

Participating laboratories will be capable of the following:

1. Providing Standard Operating Procedures (SOPs, see **Section 1.4**) for the performance of the BG1LUC4E2 ER TA agonist and antagonist assays
2. Conducting the study in accordance with or in the spirit of Good Laboratory Practices (GLP)
3. Providing study reports and all associated data from studies outlined in this document to the Study Management Team (SMT) through the designated contacts listed in **Section 2.2**.

1.3 Guidelines

The Project Officer and designated members of the SMT may inspect participating laboratory testing facilities and audit any procedures. participating laboratories should notify the SMT of any changes in Key Personnel (see **Section 3.1.1**)

1.4 Definitions

Good Laboratory Practices (GLPs): Regulations governing the conduct, procedures, and operations of toxicology laboratories developed to assure the quality and integrity of the data and to address such matters as organization and personnel, facilities, equipment, facility operations, and study conduct (OECD, 1998).

Standard Operating Procedures (SOPs): Written documents that describe in sufficient detail the routine procedures to be followed for a specific operation, analysis, or action. Consistent use of an approved SOP ensures conformance with organizational practices; reduced work effort; reduction in error

occurrences; and improved data comparability, credibility, and defensibility, SOPs also serve as resources for training and for ready reference and documentation of proper procedures.

Study Design and Work Plan: A description of all phases of the validation study and the purpose of the procedures; also provides guidance for the preparation of reports.

Test Method Protocols: Specific and detailed guides for performing the BG1LUC4E2 ER TA assay for the detection of ER agonists and antagonists.

Test Substances: Chemicals supplied to participating laboratories that are coded and distributed such that only the Project Officer, the SMT, and the Substance Inventory and Distribution Management (identified in **Section 2.2.2**) have knowledge of the identity of each test substance. The test substances will be purchased, aliquoted, coded, and distributed by the Substance Inventory and Distribution Management, under the guidance of the Project Officer and the SMT.

2.0 ORGANIZATION

2.1 Validation Study Sponsors

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)
The European Centre for the Validation of Alternative Methods (ECVAM)
The Japanese Center for the Validation of Alternative Methods (JaCVAM)

2.2 Study Management

2.2.1 International Study Management Team

2.2.1.1 *NICEATM*

Dr. William Stokes (NICEATM/NIEHS) – Co-Chair/Project Officer
Dr. Raymond Tice (NICEATM/NIEHS) – Co-Chair
Dr. David Allen (NICEATM/ILS) – NICEATM Principal Investigator
Mr. Frank Deal (NICEATM/ILS) – Project Coordinator
Ms. Patricia Ceger (NICEATM/ILS) – Assistant Project Coordinator
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P.O. Box 12233
Research Triangle Park, NC 27709

2.2.1.2 *ECVAM*

Dr. Susanne Bremer
Dr. Miriam Jacobs
Mailing Address:
Joint Research Center – European Commission
21020 Ispra (VA), Italy

2.2.1.3 *JaCVAM*

Dr. Hajime Kojima
Dr. Atsushi Ono
Mailing Address:
National Institute of Health Sciences
Kamiyouga 1-18-1, Setagaya-ku, Tokyo 158-8501, Japan

2.2.2 Substance Inventory and Distribution Management

Dr. Cynthia Smith
Chemistry Resources Group Leader
Mailing Address:
NIEHS
111 Alexander Dr.
Research Triangle Park, NC 27709

3.0 TESTING FACILITY AND KEY PERSONNEL

3.1 Competence and Capabilities

Participating laboratories should be competent in the conduct of the BG1LUC4E2 ER TA assay and will provide competent personnel, adequate facilities, equipment, supplies, proper health and safety guidelines, and quality assurance procedures.

3.1.1 Personnel

3.1.1.1 *Facility Management*

Participating laboratory facility management is responsible for establishing scientific guidelines and procedures, training and supervision of technical staff, and evaluation of results. The facility manager must maintain training files that include qualifications, experience, and a job description for each individual involved in the BG1LUC4E2 ER TA assay validation study.

3.1.1.2 *Study Director*

The Study Director has the overall responsibility for the BG1LUC4E2 ER TA assay validation study conducted at each participating laboratory. The Study Director should be responsible for providing Standard Operating Procedures (SOPs) for use during the validation study.

3.1.1.3 *Quality Assurance (QA) Personnel*

Participating laboratory QA personnel or SMT sponsored reviewer (independent reviewer) should monitor the validation study to assure compliance with good laboratory practices for all aspects of the validation study.

3.1.1.4 *Consultant(s)*

Consultants are scientists or other professionals of appropriate education, training, and experience with the BG1LUC4E2 ER TA assay who provide scientific guidance to the SMT or participating laboratories.

3.1.1.5 *Laboratory Technician(s)*

Each individual engaged in the conduct of or responsible for the supervision of the assay should have education, training, and experience, or combination thereof, to enable that individual to perform the assigned duties.

3.1.1.6 *Safety Officer*

A designated Safety Officer (someone not involved in the actual conduct of the validation study) will receive the blinded (coded) test substances from Substance Inventory and Distribution Management and transfer the substances to the Study Director. A sealed health and safety information package will accompany the coded test substances and the Safety Officer should retain the package until the completion of the validation study. The Safety Officer will promptly notify the SMT Project Coordinator if this is opened at any time during the validation study.

3.1.2 Facilities, Equipment, and Supplies

3.1.2.1 *Cell Culture Laboratory*

A designated cell culture laboratory should be available to ensure that the BG1LUC4E2 ER TA assay can be performed using good cell culture practice (Coecke et al. 2005). Access to the validation study assays and reference substances should be restricted to appropriate personnel as determined by participating laboratory management.

3.1.2.2 *Equipment*

Equipment that is required for conducting the BG1LUC4E2 ER TA agonist and antagonist assays, as specified in the BG1LUC4E2 ER TA assay ER agonist and antagonist protocols. All equipment maintenance and calibration should be routinely performed and documented.

3.1.3 Health and Safety

Participating laboratories should conform to all relevant health and safety regulations in the conduct of the validation study. The designated Safety Officer should be the point of contact for health and safety issues.

3.1.4 Quality Assurance

Participating should conduct this validation study in compliance with or in the spirit of Good Laboratory Practice (GLP) Standards (OECD 1998). QA personnel from the participating or representing the SMT should review the protocol and audit the in-life phase, study workbook, and final report data.
The final reports for all phases of the validation study should be audited by QA personnel for GLP compliance and a QA Statement should be provided with each final report. Each final report should identify: 1) the phases and data inspected, 2) the dates of inspection, and 3) the dates findings were reported to the Study Director and participating laboratory management. The QA Statement should identify whether the methods and results described in the final report accurately reflect the raw data produced during the validation study.

4.0 TEST PHASES AND SCHEDULE

4.1 Study Timeline and Deliverables

4.1.1 Study Timeline

TASK	ACTIVITIES	TIMELINE
Phase I	• Development of automated testing procedures (XDS) • Qualification/protocol refinement by testing reference standards and controls • Establish historical database for standards and controls by conducting independent experiments (10 each for the agonist and antagonist protocols) • Submission of draft report and review by SMT	Mar. 07 – Jul. 07
Phase IIa	• Four substances from ER minimum list tested independently three times for agonism and antagonism (24 total experiments to include the quantitative assessment of cell viability in parallel plates in the agonist and antagonist assays) • Submission of draft report and review by SMT	Aug. 07 – Sep. 07
Phase IIb	• Eight substances from ER minimum list tested independently three times (48 total experiments) • Submission of draft report and review by SMT	Oct. 07 – Dec. 07
Phase III	• Remaining 41 substances from ER minimum list tested once for agonism and antagonism (82 total experiments) • Submission of draft report and review by SMT	Jan. 08 – Feb. 08
Phase IV	• Testing of remaining 25 substances from ER list for agonism and antagonism (XDS only), (50 total experiments) • Submission of draft report and review by SMT	Mar. 08

4.1.2 Study Deliverables

4.1.2.1 *Test Results (Phases I-IV)*

Participating laboratories will provide raw and quality control data in electronic format (i.e., email with attachments) to the SMT Project Coordinator on a weekly basis during *in-life* (i.e., during those weeks when BG1LUC4E2 ER TA assay data is being collected and/or analyzed) portions of the study.

4.1.2.2 *Study Status Reports (Phases I-IV)*

Participating laboratories will provide study status reports during each phase of the study to the SMT Project Coordinator on a biweekly basis. These reports will be provided in electronic format (i.e., email with attachments) and will include raw and quality control data as the study progresses. Reports should contain the information outlined in **Appendix A**.

4.1.2.3 *Draft Reports (Phases I-IV)*

At the conclusion of each phase of the study, a draft report will be provided by the Study Director to the SMT Project Coordinator. The draft report will be provided electronically in Word®. Reports should contain the information outlined in **Appendix A** and should follow the recommended formats and styles provided in the "Style Guide for BG1LUC4E2 ER TA Validation Study Laboratory Reports and Documents" (**Appendix B**).

4.1.2.4 *Final Reports (Phases I-IV)*

Each draft report that is approved by the SMT will be followed by a final report, which has been reviewed by the QA for GLP compliance, for each phase of the study. The final report will be provided electronically in Word® by the Study Director to the SMT Project Coordinator. Copies of the audited Study Workbook pages should submitted in electronic format (i.e., pdf files) as an attachment to the report. However, completion of the final report is not required prior to initiation of the next phase of the validation study.

4.1.3 Estimated Due Dates for Reports

ESTIMATED DUE DATES					
REPORTS	**PHASE I**	**PHASE IIa**	**PHASE IIb**	**PHASE III**	**PHASE IV**
Study Status	*	*	*	*	*
Draft	Jul., 2007	Sep., 2007	Dec., 2007	Feb, 2008	Mar., 2008
Final	Aug., 2007	Oct., 2007	Jan., 2008	Mar, 2008	Apr., 2008

*Study status reports will be provided biweekly during each phase of the study.

4.2 Phase I

This phase will be used for initial laboratory qualification/protocol refinement by all participating laboratories and is limited to the testing of reference standards, positive controls, and the solvent control. The results will be used to establish an historical database in each laboratory for reference standards and controls.

4.2.1 Initial Laboratory Qualification/Protocol Refinement

Repetitive testing of agonist and antagonist reference standards and positive/solvent controls will be used to demonstrate proficiency with the BG1LUC4E2 ER TA assay, demonstrate intralaboratory repeatability and intra- and inter-laboratory reproducibility, and establish an historical database. Results will be compared to historical control data established during the BG1LUC4E2 ER TA Protocol Standardization Study. If there is excessive variation of reference standard and control data within or among the participating laboratories, the SMT (through the designated contacts) will work with the laboratories to determine cause and recommend appropriate actions needed to reduce variation. Statements of Work, Test Method Protocols, and SOPs will be revised, if necessary, and testing repeated until acceptable proficiency is demonstrated (i.e., acceptable intralaboratory repeatability and intra- and inter-laboratory reproducibility). The SMT may convene a teleconference with appropriate participants of the validation study to discuss information concerning the progression of the validation study.

4.2.2 Criteria for Advancing to Phase II

The SMT will decide when all laboratories will advance to Phase II of the validation study, based on the following criteria:

- Data, reviewed by the participating laboratory QA personnel (or independent reviewer), has been received by the SMT
- All participating laboratories have submitted acceptable draft reports as outlined in **Section 4.1.2.2**.

- Acceptable intralaboratory repeatability and intra- and inter-laboratory reproducibility has been demonstrated by the participating laboratories
- A suitable historical negative and positive control database has been established

4.3 Phase II

Phase II provides for initial laboratory qualification using procedures that have been refined in Phase I, but is also the initial phase for testing substances from the ICCVAM list of 78 reference substances recommended for validation of ER TA assays. In this phase, four coded test substances (Phase IIa) and then eight coded test substances (Phase IIb) will be tested in all three participating laboratories. Acceptance criteria for experimental data for Phase IIa will be based on the historical database established in Phase I for reference standards and controls. Reference standard and control data collected during Phase IIa will also be included in the historical database, which will then be used to establish acceptance criteria for Phase IIb.

4.3.1 Phase IIa Limited Testing of Protocol and Protocol Refinement

After a range-finding assay is completed for each of the four coded test substances in Phase IIa, recommended starting concentrations for the comprehensive concentration-response experiment and the rationale for their selection are to be sent to the SMT for review and approval. The comprehensive concentration-response experiment for each test substance should not begin until the starting concentrations have been approved, and they should not be modified without approval from the SMT. The comprehensive concentration-response experiment should be performed three times, once on each of three different days. Laboratories will calculate EC_{50} values for the agonist reference standard or IC_{50} values for the antagonist reference standard (in µg/mL). Laboratories will also calculate EC_{50} or IC_{50} values (in µg/mL), when possible, for coded test substances. These data, along with all quality control, raw, derived and supporting data, will be reported to the SMT through the designated contacts. If there is excessive variation within or among participating laboratories, the SMT will work with the laboratories to determine the cause and recommend appropriate actions needed to reduce variation. Statements of Work, Test Method Protocols, and SOPs will be revised, if necessary, and testing repeated until acceptable proficiency is demonstrated (i.e., acceptable intralaboratory repeatability and intra- and inter-laboratory reproducibility). The SMT may convene a teleconference with appropriate participants of the validation study to discuss information concerning the progression of the validation study.

4.3.2 Criteria for Advancing to Phase IIb

The SMT will decide when all laboratories will advance to the Phase IIb of the validation study, based on the following criteria:

- Data, reviewed by the QA Officer (or independent reviewer), has been received by the SMT
- All participating laboratories have submitted acceptable draft reports as outlined in **Section 4.1.2.2**.
- Acceptable intralaboratory repeatability and intra- and inter-laboratory reproducibility has been demonstrated by the participating laboratories

4.3.3 Phase IIb Testing of Protocol and Protocol Refinement

Phase IIb includes the testing of eight coded substances and is the last phase for evaluating any protocol refinements from Phase I or IIA.

After a range-finding assay is completed for each of the eight coded test substances in Phase IIb, recommended starting concentrations for the comprehensive concentration-response experiment and the rationale for their selection are to be sent to the SMT for review and approval. The comprehensive concentration-response experiment for each test substance should not begin until the starting concentrations have been approved and should not be modified without approval of the SMT. The comprehensive concentration-response experiment should be performed three times, once on each of three different days. Laboratories will calculate EC_{50} values for the agonist reference standard or IC_{50} values for the antagonist reference standard (in µg/mL). Laboratories will also calculate EC_{50} or IC_{50} values (in µg/mL), when possible, for coded test substances. These data, along with all quality control, raw, derived and supporting data, will be reported to the SMT through the designated contacts. If there is excessive variation within or among participating laboratories, the SMT will work with the laboratories to determine the cause and recommend appropriate actions needed to reduce variation. Statements of Work, Test Method Protocols, and SOPs will be revised, if necessary, and testing repeated until acceptable proficiency is demonstrated (i.e., acceptable intralaboratory repeatability and intra- and inter-laboratory reproducibility). The SMT may convene a teleconference with appropriate participants of the validation study to discuss information concerning the progression of the validation study.

4.3.4 Criteria for Advancing to Phase III

The SMT will decide when all laboratories will advance to the Phase III of the validation study, based on the following criteria:

- Data, reviewed by participating laboratory QA personnel (or independent reviewer), has been received by the SMT
- All participating laboratories have submitted acceptable draft reports as outlined in **Section 4.1.2.2**.
- Acceptable intralaboratory repeatability and intra- and inter-laboratory reproducibility has been demonstrated within and among the participating laboratories

4.4 Phase III

In Phase III, a subset of 41 substances from the ICCVAM list of 78 recommended reference substances for validation of ER TA assays will be tested in each laboratory to evaluate interlaboratory reproducibility. Reference standard and control data collected during Phase IIb will be added to the historical database compiled in Phases I and IIa and this combined historical database will be used to establish acceptance criteria for Phase III.

4.4.1 Phase III Testing

After a range-finding assay is completed for each of the 41 coded test substances, recommended starting concentrations for the comprehensive concentration-response experiment and the rationale for their selection are to be sent to the SMT for review and approval. The comprehensive concentration-response experiment for each substance should not begin until the starting concentrations have been approved and should not be modified without approval of the SMT. The comprehensive concentration-response experiment for each coded test substance should be performed once. Laboratories will calculate EC_{50}

values for the agonist reference standard or IC_{50} values for the antagonist reference standard (in µg/mL). Laboratories will also calculate EC_{50} or IC_{50} values (in µg/mL), when possible, for coded test substances. These data, along with all quality control, raw, derived and supporting data, will be reported to the SMT through the designated contacts. If there is excessive variation among participating laboratories, the SMT will work with the laboratories to determine the cause and recommend appropriate actions needed to reduce variation. Statements of Work, Test Method Protocols, and SOPs will be revised, if necessary, and testing repeated until acceptable proficiency is demonstrated (i.e., acceptable interlaboratory reproducibility). The SMT may convene a teleconference with appropriate participants of the validation study to discuss information concerning the progression of the validation study.

4.4.2 Criteria for Advancing to Phase IV

The SMT will decide when XDS will advance to Phase IV of the validation study, based on the following criteria:

- All participating laboratories have submitted acceptable draft reports as outlined in **Section 4.1.2.2**.
- Data, reviewed by QA, has been received by the SMT
- Acceptable interlaboratory reproducibility has been demonstrated among the participating laboratories

4.5 Phase IV

In Phase IV, the U.S, participating laboratory, Xenobiotic Detection Systems, Inc. (XDS) only will test the remaining 25 substances from the ICCVAM list of 78 recommended reference substances for validation of ER TA assays.

4.5.1 Phase IV Testing of Remaining ICCVAM Substances

After a range-finding assay is completed for each of the remaining 25 coded test substances, recommended starting concentrations for the comprehensive concentration response experiments and the rationale for their selection are to be sent to the SMT for review and approval. The comprehensive concentration-response experiment for each substance should not begin until the starting concentrations have been approved and should not be modified without approval of the SMT. The comprehensive concentration-response experiment for each coded test substance should be performed once. XDS will calculate EC_{50} or IC_{50} values (in µg/mL) for reference standards and coded test substances, and report this and all raw, derived, and supporting data to the SMT Project Coordinator.

4.5.2 Criteria for Completion of Phase IV

Phase IV will be considered complete once all of the remaining 25 coded substances have been tested, data reviewed by QA has been received by the SMT, and the Study Director provides a final report to the SMT Project Coordinator.

5.0 REFERENCE STANDARDS, CONTROLS AND TEST SUBSTANCES

Substance Inventory and Distribution Management (see **Section 2.2.2**) will supply all reference standards and control substances for the validation study, which will be shipped prior to initiation of testing. Phase IIa coded test substances will be shipped as a unit of eight (four substances for testing in the agonist

protocol and four substances for testing in the antagonist protocol). Phase IIb coded test substances will be shipped as a unit of 16 (eight substances for testing in the agonist protocol and eight substances for testing in the antagonist protocol). Phase III coded test substances will be shipped as a unit of 82 (41 substances for testing in the agonist protocol and 41 substances for testing in the antagonist protocol) and Phase IV coded test substances will be shipped as a unit of 50 (25 substances for testing in the agonist protocol and 25 substances for testing in antagonist protocol). The SMT and Substance Inventory and Distribution Management will have all descriptive information for each substance (e.g., purity, Chemical Abstracts Service Registry Number® [CASRN], supplier, etc.).

5.1 Reference Substances

5.1.1 Range of Responses

The substances proposed for the validation study are representative of a range of ER TA responses, chemical classes, and physico-chemical properties.

5.1.2 Receipt of Reference Standards, Controls, and Test Substances

Reference standards, controls, and test substances will be packaged so as to minimize damage during transit and will be shipped according to proper regulatory procedures. Coded test substances will be packaged and shipped so as to conceal their identities. Each participating laboratory and the SMT will be notified by Substance Inventory and Distribution Management when any reference standards, controls, and test substances are shipped.

Upon receipt, substances should be stored in appropriate storage conditions as per recommendations provided by Substance Inventory and Distribution Management. Each participating laboratory should notify the SMT Project Coordinator upon receipt of the reference substances. Coded test substances, along with a sealed health and safety information package will be shipped to the designated Safety Officer. The Safety Officer should retain the safety information package and pass the coded test substances to the Study Director. The safety information package will contain necessary information about the substance hazards and provide instructions for emergency actions. A disclosure key for identifying the test substances by code will also be included in the package. If the health and safety package must be opened during the course of the validation study (see **Section 5.5**), the Safety Officer should immediately notify the SMT Project Coordinator.

5.1.3 Test Substance Information for the Study Director

Before shipping coded test substances, the SMT Project Coordinator will send the Study Director data sheets containing a minimum of essential information, including color, physical state, weight or volume of sample, specific density for liquid reference substances, and storage instructions to the Study Director.

5.2 Control Materials

The solvent control for both agonist and antagonist assays is 1.0% dimethyl sulfoxide (DMSO) in cell culture medium.

5.2.1 Positive Control (PC)

5.2.1.1 *Agonist Assay (PC)*

Methoxychlor (CASRN: 72-43-5) (3.13 μg/mL) is used as the agonist positive control for all comprehensive concentration-response tests for agonism.

5.2.1.2 *Antagonist Assay (PC)*

Flavone (CASRN: 525-82-6) (25 μg/mL) is used as the antagonist positive control for all comprehensive concentration-response tests for antagonism.

To demonstrate antagonism, a fixed concentration of estradiol (CASRN: 50-28-2) (2.5×10^{-5} μg/mL) is included as a control in all range finding and comprehensive concentration-response tests for antagonism.

5.2.2 Reference Standards

5.2.2.1 *Agonist Assay*

Estradiol (CASRN: 50-28-2) is used as the reference standard for agonist testing, run at 3 different concentrations for range finding and as an 10-point 2-fold serial dilution for comprehensive concentration-response testing.

5.2.2.2 *Antagonist Assay*

Estradiol (CASRN 50-28-2) (1.25×10^{-5} μg/mL) and raloxifene (CASRN 84449-90-1) run at 3 different concentrations for range finding and as a 9-point 2-fold serial dilution for comprehensive concentration-response testing is used as the reference standard for antagonist testing.

5.3 Inventory of Test Substances

The amount of test substance received, the amount used for specific tests, and the amount remaining should be documented by the participating laboratory.

5.4 Disposition of Test Substances

After the studies are completed, any remaining substance will be returned to Substance Inventory and Distribution Management or appropriately disposed of by the participating laboratory.

5.5 Handling of Test Substances

Appropriate safety procedures should be followed in handling the test substances. Personnel should be instructed to treat all test substances as ***very hazardous and potentially carcinogenic*** and to properly dispose of laboratory wastes as toxic wastes. The health and safety information package provided to the facility Safety Officer should be opened only during an emergency situation.

6.0 TEST SYSTEM

All testing procedures and data analyses should follow the Test Method Protocols (**Appendices B** and **C**) and Statement of Work provided by the SMT.

7.0 DATA COLLECTION

7.1 Nature of Data to be Collected

Both raw and summary data from experiments performed under this Statement of Work should be provided to the SMT via the SMT Project Coordinator.

7.2 Type of Media Used for Data Storage

All raw data should be collected and archived at the end of the study (under the direction of the Study Director). Backup files should be produced and maintained for data that are stored electronically.

7.3 Documentation

Raw data include, but are not limited to the following:

a) data recorded in the Study Workbook, which should consist of recordings of all activities related to preparing the BG1LUC4E2 ER TA TA agonist and antagonist reference standards, controls and test substances, and performing the agonist and antagonist assays
b) computer printouts of luminometer data
c) equipment logs
d) equipment calibration records
e) test substance logs
f) cryogenic freezer inventory logs
g) cell culture media preparation logs

8.0 VALIDATION STUDY PHASE DRAFT AND FINAL REPORTS

As noted in **Section 4.1.2.2**, a draft report should be submitted to the SMT Project Coordinator at the completion of each study phase (i.e., Phases I, IIa, IIb, III, and IV). Once the draft reports are accepted, a final report for each study phase should be prepared, signed by the Study Director and accompanied by a signed Quality Assurance Statement, and provided to the SMT Project Coordinator following acceptance of the corresponding draft report. See **Appendix A** for recommended phase-specific report contents and **Appendix D** for recommended report formats and styles.

9.0 RECORDS AND ARCHIVES

At the end of the validation study, the original raw and derived assay data, as well as copies of other raw data not exclusive to this validation study (instrument logs, calibration records, facility logs, etc.), should be stored and archived for at least five years. At the end of this five year-storage and archiving period, these stored/archived materials should be submitted to NICEATM for storage and archiving.

10.0 SUPPORTING DOCUMENTS

Coecke S, Balls M, Bowe G, Davis J, Gstraunthaler G, Hartung T, Hay R, Merten O, Price A, Schectman L, Stacey G, Stokes W. 2005. Guidance on Good Cell Culture Practice: A Report of the Second ECVAM Task Force on Good Cell Culture Practice. ATLA 33:261-287.

Federal Register (FR) Notice (Vol. 71, No. 51, pp. 13597-13598, March 16, 2006): Notice of Availability of a Revised List of Recommended Reference Substances for Validation of *In Vitro* Estrogen and Androgen Receptor Binding and Transcriptional Activation Assays: Request for Comments and Submission of *In Vivo* and *In Vitro* Data. Available: http://iccvam.niehs.nih.gov/docs/FR/frnotice.htm [accessed 24 March 2006]

ICCVAM. 2002. Expert Panel Evaluation of the Validation Status of *In Vitro* Test Methods for Detecting Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays - Expert Panel Final Report. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/docs.htm [accessed 24 March 2006]

ICCVAM. 2003. ICCVAM Evaluation of *In Vitro* Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays. NIH Pub. No. 03-4503. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/endocrine.htm [accessed 14 February 2006]

OECD. 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring Number 1: OECD principles on Good Laboratory Practice. [as revised in 1997]. ENV/MC/CHEM[98]17. Paris: OECD

APPENDIX A

RECOMMENDED REPORT CONTENTS

STUDY STATUS REPORTS
BG1LUC4E2 ER TA Validation Study – Phases I – IV

Report Date:

Substances Received:
Study status reports should include information on standards and controls received, with the information for those substances presented in tabular format as per **Table A-1**.

Table A-1 Substance Receipt Reporting Template for BG1LUC4E2 ER TA Validation Study

XDS Identification Number	Sponsor Identification Number	Physical Description	Storage Conditions	Receipt Date	Received By	Comments

If no test substances were received during the time period described in the report, indicate "no test substances or controls received."

Range Finding Results:

Study status reports for range finding results should include:

- Information regarding any problems with test substance solubility in DMSO or 1% DMSO/aqueous cell culture media that prevented the conduct of experiments at the limit dose (1000 µg/mL) specified in the BG1LUC4E2 ER TA assay protocols in **Appendices B** and **C**
- The number of range finder experiments performed during the time period described in the study status report. If no range finder experiments were conducted during this time, indicate "no range finder experiments conducted"
- Excel® spreadsheets of range finder data as described in BG1LUC4E2 ER TA assay protocols in **Appendices B** and **C**

- Graphs of range finder results as per **Figures A-1** and **A-2** using instructions in the provided NICEATM Prism® Users Guide
- The recommended starting concentration for the comprehensive concentration-response experiments for each test substance and the rationale for its use

Comprehensive Concentration-Response Testing Results:

Study status reports for comprehensive concentration-response testing results should include:

- The number of comprehensive experiments performed during the time period described in the study status report. If no comprehensive experiments were conducted during this time, indicate "no comprehensive experiments conducted".
- Excel® spreadsheets of data as described in BG1LUC4E2 ER TA assay protocols in **Appendices B** and **C**.
- Graphs of results as per **Figures A-3** and **A-4** using instructions in the provided NICEATM Prism® Users Guide.

Problems Encountered:

List any problems encountered during range finder, cytotoxicity, and/or comprehensive testing, and their resolution.

Other Information: (All copies of printouts, documents, and spreadsheets will be noted as exact duplicates of the data):

- Copies of raw data generated with the spectrophotometric plate reader
- Copies of completed Microsoft® Excel spreadsheets and Prism® files used for data collection and determination of the EC_{50} or IC_{50} values for the reference standard.
- Copies of the protocols
- Deviations to the protocols, SOPs, and/or Statement of Work

Projected Activities and Schedule:

Provide an estimate of the number and type of experiments (e.g., range finder or comprehensive experiments) to be conducted during the next biweekly study status reporting period. If no experiments will be performed, indicate that no experiments will be conducted.

APPENDIX A (cont.)

RECOMMENDED REPORT CONTENTS

DRAFT/FINAL REPORT NO. 1

BG1LUC4E2 ER TA Validation Study – Phase I

TITLE PAGE
Study Title: Draft/Final Report 1: BG1LUC4E2 ER TA Validation Study – Phase 1
Authors:
Testing Facility: Name and address
Experimental Start Date: The date on which the first phase specific data are collected.
Experimental End Date: The last date on which phase specific data are collected.
Archive Location: Name and address
Study Director: Name
Key Personnel: Laboratory technicians, QA Director, Safety Officer, Facility Manager
Scientific Advisor (if applicable): Name

QUALITY ASSURANCE STATEMENT (Final Reports Only)
The final reports for all phases of the validation study should be accompanied by a signed QA Statement that includes: 1) the phases and data inspected, 2) the dates of inspection, and 3) the dates findings were reported to the Study Director and laboratory management. The QA Statement should identify whether the methods and results described in the final report accurately reflect the raw data produced during the validation study.

TABLE OF CONTENTS
The Table of Contents should be formatted as specified by the provided "Style Guide for BG1LUC4E2 ER TA Validation Study" (**Appendix D**).

EXECUTIVE SUMMARY
The executive summary should state the specific objectives of Phase I and review the experimental procedures and results that support the achievement of the objectives.

METHODS

A description of the protocol elements used for generation and analysis of data should be provided. This should also include information on standards and controls received, and be presented in tabular format as per **Table A-1**.

RESULTS

This section of Phase I should include a table containing the results from all experiments performed during Phase I as per **Table A-2**. This section should also include graphical representations of the data collected during the compilation of the historical database using instructions from the provided NICEATM Prism Users Guide as follows:

- Agonist Quality Controls
    - a graph depicting the combined results for the methoxychlor control
    - a graph depicting the combined results for the DMSO control
    - a graph depicting the combined results for the fold induction of the E2 reference standard
    - a graph depicting the combined EC_{50} values of the E2 reference standard
- Antagonist Quality Controls
    - a graph depicting the combined results for the flavone control
    - a graph depicting the combined results for the DMSO control
    - a graph depicting the combined results for the fold reduction of the Ral/E2 reference standard
    - a graph depicting the combined IC_{50} values of the Ral/E2 reference standard

DISCUSSION

Results, including a description of any problems that were encountered and how they were resolved, should be presented and discussed.

SIGNATURE PAGE

Study Director: Name, signature and date

Table A-2 Example Summary of Experiments Template

Experiments: Phase I						
Experiment I.D.	**Substance Code**	**Date**	**Plate Induction[1]**	**EC_{50} (µg/mL)[2]**	**Experiment Used for Data Analysis or Repeated**	**Reason Why Experiment Not Used**
AG1	E2	09/16/05	not calculated	not calculated	Repeated	Induction not ≥ to 3 fold
AG2	E2	09/16/05	not calculated	not calculated	Repeated	Positive control greater than historical mean plus 2.5 times the SD.
AG3	E2	09/16/05	not calculated	not calculated	Repeated	Plate was dropped
AG4	E2	09/23/05	8.4	2.95E-11	Used	N/A
AG5	E2	09/23/05	12.6	1.98E-11	Used	N/A
AG6	E2	09/29/05	7.4	1.95E-11	Used	N/A
AG7	E2	09/30/05	8.6	2.05E-11	Used	N/A
AG8	E2	10/06/05	6.5	2.35E-11	Used	N/A
AG9	E2	10/12/05	8.9	2.58E-11	Used	N/A
AG1-Repeat1	E2	10/12/05	9.9	2.90E-11	Used	N/A

[71] Column heading is "Plate Induction" for agonist testing and "Plate Reduction" for antagonist testing

[2] Column heading is "EC_{50}" for agonist testing and "IC_{50}" for antagonist testing

APPENDIX A (cont.)

RECOMMENDED REPORT CONTENTS

DRAFT/FINAL REPORTS NO. 2-5
BG1LUC4E2 ER TA Validation Study – Phases II - IV

TITLE PAGE
Study Title:
Draft/Final Report 2: BG1LUC4E2 ER TA Validation Study – Phase I1a
Draft/Final Report 3: BG1LUC4E2 ER TA Validation Study – Phase 1Ib
Draft/Final Report 4: BG1LUC4E2 ER TA Validation Study – Phase III
Draft/Final Report 5: BG1LUC4E2 ER TA Validation Study – Phase IV
Authors:
Testing Facility: Name and address
Experimental Start Date: The date on which the first phase specific data are collected.
Experimental End Date: The last date on which phase specific data are collected.
Archive Location: Name and address
Study Director: Name
Key Personnel: Laboratory technicians, QA Director, Safety Officer, Facility Manager
Scientific Advisor (if applicable): Name

QUALITY ASSURANCE STATEMENT (Final Reports Only)
The final reports for all phases of the validation study should be accompanied by a signed QA Statement that includes: 1) the phases and data inspected, 2) the dates of inspection, and 3) the dates findings were reported to the Study Director and laboratory management. The QA Statement should identify whether the methods and results described in the final report accurately reflect the raw data produced during the validation study.

TABLE OF CONTENTS
The Table of Contents should be formatted as specified by the provided "Style Guide for BG1LUC4E2 ER TA Validation Study" (**Appendix D**).

EXECUTIVE SUMMARY
The summary should state the specific objectives of Phases II to IV and review the experimental procedures and results that support the achievement of the objectives.

METHODS
A description of the protocol elements used for generation and analysis of data should be provided. This section should include information on coded test substances received as per **Table A-1**.

RESULTS

Range Finding:

The results section relevant to the range finding experiments conducted in Phases II to IV should include the following:

- Information regarding any issues with test substance solubility in DMSO or 1% DMSO/aqueous cell culture media that prevented the conduct of experiments at the limit dose (1.0×10^3 µg/mL) specified in the BG1LUC4E2 ER TA assay protocols in **Appendices B** and **C**
- A table indicating the concentrations tested and the cell viability results for each concentration tested as per **Table A-3**
- A table containing all phase specific range finding experiments performed during the Phase as per **Table A-4**
- Graphical representation of range finding results for each test substance experiment as per **Figures A-1** and **A-2** using instructions from the provided NICEATM Prism® Users Guide
- The recommended starting concentration for comprehensive concentration-response experiment for each test substance and the rationale for its use

Table A-3 Example Table for Range Finding Concentrations Tested and Cell Viability

Substance Code	Concentrations Tested (µg/mL)	Cell Viability Results
V0001	$1.00 \times 10^{+2}$	
	$1.00 \times 10^{+1}$	
	$1.00 \times 10^{+0}$	
	1.00×10^{-1}	
	1.00×10^{-2}	
	1.00×10^{-3}	
V0002	$1.00 \times 10^{+2}$	
	$1.00 \times 10^{+1}$	
	$1.00 \times 10^{+0}$	
	1.00×10^{-1}	
	1.00×10^{-2}	
	1.00×10^{-3}	

Table A-4 Example Summary of Experiments Template: Range Finder Testing

Experiments: Phase IIa Range Finder Testing						
Experiment I.D.	**Substance Code**	**Date**	**Plate Induction[1]**	**EC_{50} (µg/mL)[2]**	**Experiment Used for Data Analysis or Repeated?**	**Rationale for Unacceptability**
RF 1	V0001	09/16/05	9.1	2.94E-11	Used	Acceptable
RF 2	V0002	09/16/05	8.9	2.92E-11	Used	Acceptable
RF 3	V0003	09/16/05	2	not calculated	Repeated	Induction too low
RF 4	V0004	09/23/05	9.3	2.98E-11	Used	Acceptable
RF3-Repeat	V0003	10/12/05	9.9	2.90E-11	Used	Acceptable

[1] Column heading is "Plate Induction" for agonist testing and "Plate Reduction" for antagonist testing

[2] Column heading is "EC_{50}" for agonist testing and "IC_{50}" for antagonist testing

Figure A-1 Example Agonist Range Finder Results Graph

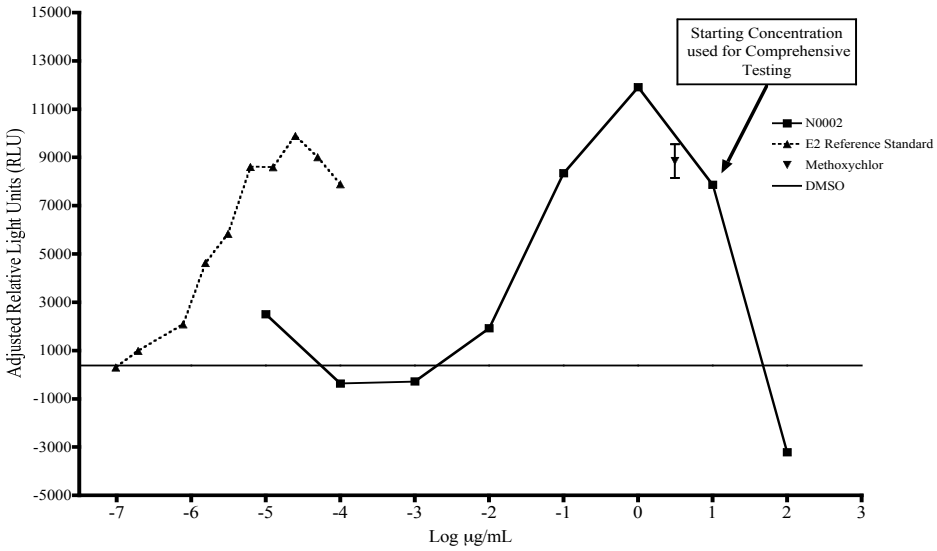
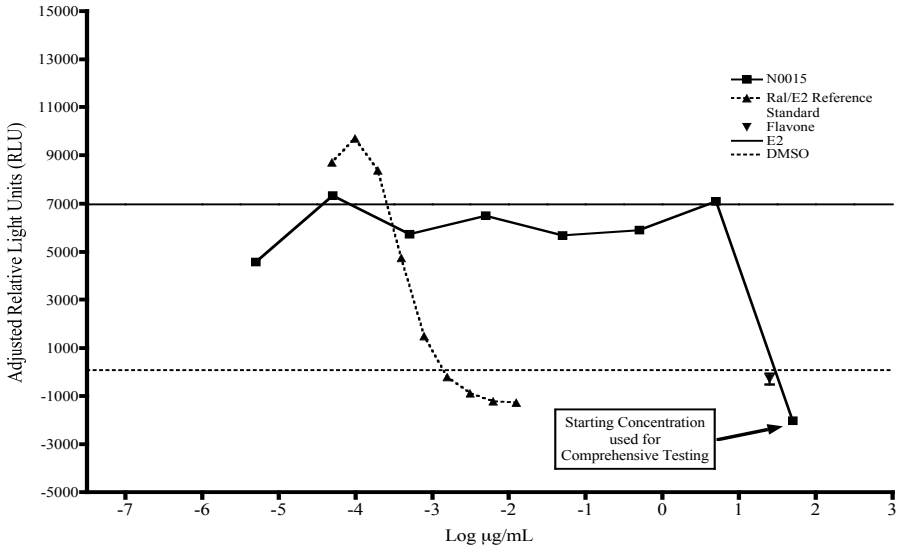


Figure A-2 Example Antagonist Range Finder Results Graph



Comprehensive Concentration Response Testing:

The results section relevant to the comprehensive concentration-response experiments conducted in Phases II-IV should include the following:

- A table indicating the concentrations tested for each substance tested during the phase and the cell viability results for each concentration tested as per **Table A-5**
- A table containing the phase specific experiments performed during the phase as per **Table A-6**
- Graphical representation of the combined results for each substance tested in the comprehensive concentration-response experiment as per **Figures A-3** and **A-4** using instructions from the provided NICEATM Prism® Users Guide

Table A-5 Example Concentrations Tested and Cell Viability Table

Substance Code	Concentrations Tested (µg/mL)	Cell Viability Results
V0001	1.00×10^{-2}	
	5.00×10^{-3}	
	2.50×10^{-3}	
	1.25×10^{-3}	
	6.25×10^{-4}	
	3.13×10^{-4}	
	1.56×10^{-4}	
	7.81×10^{-5}	
	3.91×10^{-5}	
	1.95×10^{-5}	
	9.77×10^{-6}	
V0002	5.00×10^{-3}	
	2.50×10^{-3}	
	1.25×10^{-3}	
	6.25×10^{-4}	
	3.13×10^{-4}	
	1.56×10^{-4}	
	7.81×10^{-5}	
	3.91×10^{-5}	
	1.95×10^{-5}	
	9.77×10^{-6}	
	4.89×10^{-6}	

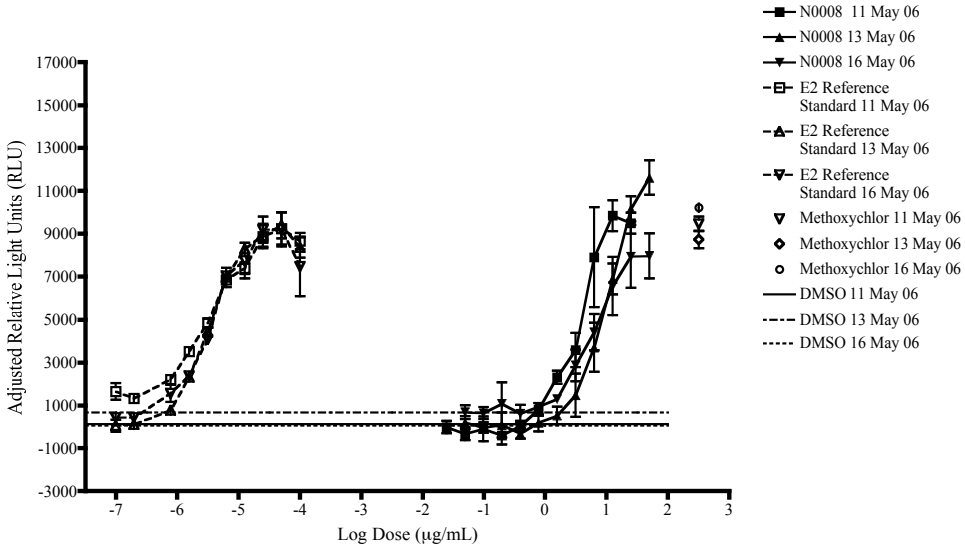
Table A-6 Example Summary of Experiments Template: Comprehensive Testing

Experiments: Phase II-IV Comprehensive Testing						
Experiment I.D.	**Substance Code**	**Date**	**Plate Induction[1]**	**EC50 (µg/mL)[2]**	**Experiment Used for Data Analysis or Repeated?**	**Rationale for Unacceptability**
CT 1	V0001	09/16/05	2	not calculated	Repeated	Induction too low.
CT 2	V0002	09/16/05	8.9	2.92E-11	Used	Acceptable
CT 3	V0003	09/16/05	9.1	2.94E-11	Used	Acceptable
CT 4	V0004	09/23/05	9.3	2.98E-11	Used	Acceptable
CT1-Repeat	V0001	10/12/05	9.9	2.90E-11	Used	Acceptable

[1] Column heading is "Plate Induction" for agonist testing and "Plate Reduction" for antagonist testing

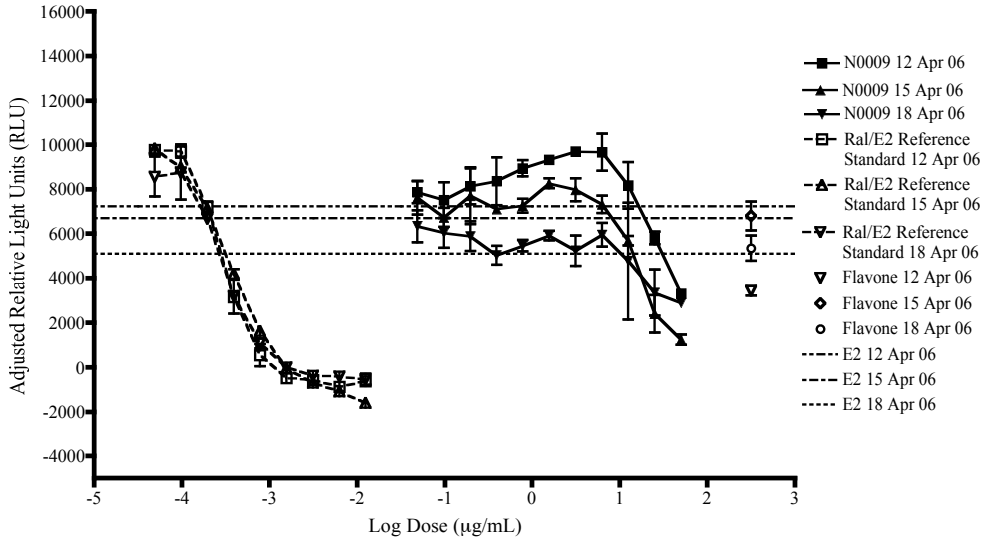
[2] Column heading is "EC_{50}" for agonist testing and "IC_{50}" for antagonist testing

Figure A-3 Agonist Comprehensive Testing for N0008[1]



[1] Line represents the mean of three E2 replicates plus three times the standard deviation of the E2 mean

Figure A-4 Antagonist Comprehensive Testing for N0009[1]



[1] Line represents the mean of three raloxifene/E2 replicates minus three times the standard deviation of the raloxifene/E2 mean

Quality Controls:

This section should include graphical representations of quality control data used for acceptance or rejection of experiments conducted during each phase using Excel® as follows:

- Agonist Quality Controls
    - a graph depicting the combined results for the methoxychlor control
    - a graph depicting the combined results for the DMSO control

- a graph depicting the combined results for the fold induction of the E2 reference standard
- a graph depicting the combined EC_{50} values of the E2 reference standard

- Antagonist Quality Controls
  - a graph depicting the combined results for the flavone control
  - a graph depicting the combined results for the DMSO control
  - a graph depicting the combined results for the fold reduction of the Ral/E2 reference standard
  - a graph depicting the combined IC_{50} values of the Ral/E2 reference standard

DISCUSSION

Results, including a description of any problems that were encountered and how they were resolved, should be presented and discussed.

SIGNATURE PAGE

Study Director: Name, signature and date

APPENDIX B

Style Guide for BG1LUC4E2 ER TA Validation Study Laboratory Reports and Documents

STYLE GUIDE FOR BG1LUC4E2 ER TA VALIDATION STUDY LABORATORY REPORTS AND DOCUMENTS

TABLE OF CONTENTS

10.0 PURPOSE AND SCOPE

The purpose of this style guide is to specify stylistic conventions and formatting details for laboratory reports and documents for the BG1LUC4E2 ER TA validation study. This guide applies to all draft documents produced for this validation effort.

11.0 GENERAL INFORMATION

For correct spelling of words or abbreviations, use the One-look Dictionary, found at: http://library.niehs.nih.gov/research/alphalist.htm.

Documents should use default stylistic conventions per: 1997. *Franklin Covey Style Guide for Business and Technical Communication, 3rd Ed.*, Salt Lake City: Franklin Covey Co.

Documents should use the default stylistic conventions per the *Instructions for Authors* for *Environmental Health Perspectives* (EHP) at http://www.ehponline.org/docs/admin/edpolicy.html.

In case of a conflict, *EHP Instructions for Authors* supersedes *Franklin Covey Style Guide.*

Items listed in this guide supersede those in the *Franklin Covey Style Guide* and *EHP Instructions for Authors*.

12.0 STYLE AND FORMATTING RULES

12.1 Acknowledgements Section (see also Front Matter)

List the names and affiliations of persons who contributed to the preparation and publication of the document. Use the format:

ACKNOWLEDGEMENTS (bold, all caps, centered on the first line of the page)

The following individuals are acknowledged for their contributions to <fill in project name> (italics, sentence case)

List names and affiliations in double column format (column width 2.9 inches; 0.5 inches space between). Single column format may be used if there are not enough people to be listed to fill a page.

Use one of these formats for the names and affiliations:

Affiliation (Institute or Organization; bold, initial caps)

Name of first person, degrees

Name of second person, degrees (plain text, single spaced)

Use this format to list multiple people with the same affiliation.

or

Name of Person, degrees (bold, initial caps)

Affiliation

Geographic Location (e.g., Research Triangle Park, NC; plain text, single spaced)

Use this format to list individuals with different affiliations.

Always use an individual's preferred or published name.

12.2 Acronyms

Spell out an acronym the first time it is used in the following document sections: preface, executive summary, table, figure, and document body. In the text immediately following the spelled out acronym, insert the acronym in parenthesis, e.g., National Institutes of Health (NIH). Everywhere else in that section, use the acronym.

12.3 Appendices

Designate appendices by letter (e.g., Appendix A, Appendix B, etc).

If an appendix has multiple parts, designate the part by number (e.g., Appendix A1, Appendix A2, etc).

Each appendix must have a cover page that contains the appendix designation and the appendix title. If the appendix has multiple parts, a table of contents listing each sub-appendix designation, title and the beginning page number for the sub-appendix must appear on the cover page. See the **Table of Contents** entry for formatting.

Format appendix page numbers with the appendix designation, followed by a hyphen, followed by the page number (e.g., if Appendix A has 100 pages, number them A-1 through A-100, even if the appendix has multiple parts.)

12.4 Bold Text (see also Emphasis, Italics)

Use bold text only for formatting textual elements as indicated in this guide (e.g., headings, table headings, etc.).

Use bold text for figure, table, or a cross-reference to a section within the text (e.g., see **Section 2.2**)

Do not use bold text for general emphasis.

12.5 Body Text (see also, Font)

Body text is 12 pt. type, times new roman font.

12.6 Bulleted and Numbered Lists

The approved bulleted and numbered list styles are shown below.

This is sample text. This is sample text. This is sample text. This is sample text. This is sample text.

- This is sample text. This is sample text. This is sample text. This is sample text. This is sample text.

- This is sample text. This is sample text. This is sample text. This is sample text. This is sample text.

1. This is sample text. This is sample text. This is sample text. This is sample text. This is sample text.

The level of a numbered list is indicated by the left indent.

The table below shows the approved indents for each bulleted and numbered list level.

List Level	Left Indent[1]	Hanging Indent[1]	Tab Stop Position[2]
1	0.7”	0.3”	1.0’
2	1.0”	0.3”	1.3”
3	1.3”	0.3”	1.6”

[1] This setting is shown in the **Paragraph** window accessed via the **Format, Paragraph** command.
[2] This setting is shown in the **Tabs** window accessed via the **Format, Tabs** command.

Other rules for lists:

If list items are complete sentences, capitalize the first word and fully punctuate.

If list items are single words, phrases, or sentence fragments, capitalize the first word but do not use a punctuation mark at the end.

12.7 Captions (see also Figures, Tables)

A figure and table title precedes the figure or table it refers to. Captions are in 12 pt. type.

Figure titles are to be short sentences describing the contents of the figure or table,
e.g., Table 1-1 Example Figure Titles for BG1LUC4E2 ER TA

Use the format **Table 1-1 Title of Table** (bold, initial caps). Do not use a period after the table number.

Left justify the caption. Set the tab and the hanging indent at 0.7 inch.

The caption number is the chapter or section number followed by a hyphen and the sequential number of the table or figure in that chapter.

12.8 Citations within the Text

Use the format in *EHP Instructions for Authors* for citations within the text.

The use of *et al.* after the name of the first author is acceptable in a citation within the text.

12.9 Color (see also, Hyperlinks)

Use only black text in a document.

Do not use colored shading.

12.10 Commas in a series

Use a comma to separate each item in a series consisting of three or more words or phrases, including the word or phrase that immediately precedes the final conjunction (e.g., This approach was modified by assuming that there are three homogeneous subgroups rather than two: strong irritants, weak irritants, and nonirritants.)

12.11 Dates (see also Headers)

Write dates in the dd/month/yyyy format (e.g., 17 March 2006).

12.12 Document Titles

Document titles are centered, bold and in all caps. They contain the report type, test method name, and phase (e.g., **DRAFT REPORT ON LUMI-CELL® ESTROGEN RECEPTOR**

TRANSCRIPTIONAL ACTIVATION TEST METHOD – PHASE I)

12.13 e.g.,

Use the format shown for the abbreviation for *for example*.

12.14 Emphasis (see Italics)

12.15 et al. (see also Citations within the Text)

In a citation within the text, do not italicize or underline et al. (e.g., Bantle et al., 1998).

12.16 Figures (see also Captions, Tables)

Preferably, place a figure immediately after the paragraph that first cites the figure. If this is not possible, place the figure as near to this paragraph as possible.

Do not place a figure in the middle of a paragraph.

Format footnotes to figures similarly to table footnotes.

12.17 Filenames

Limit filenames to *15 characters or less*.

Test phases are referenced as roman numerals, e.g., the third phase is Phase III).

Abbreviations to be used in file names:

Agonist = Ag

Antagonist = Ant

Comprehensive Testing = CT

Draft Report = DR

Final Report = FR

Range Finder Testing = RF

Files shall be named as follows:

Excel® Spreadsheets for *Range Finding* - Laboratory designator, phase of testing, type of test, agonist or antagonist testing, replicate number (e.g, for Laboratory A, phase IIb, range finder, agonist testing, replicate number 2 would be named *AIIbRFAg2*).

Excel® Spreadsheets for *Comprehensive Testing* - Laboratory designator, phase of testing, type of test, agonist or antagonist testing, replicate number (e.g, for Laboratory A, phase III, substance V0015, comprehensive testing, antagonist testing, replicate number 2 would be named *AIIIV0015CTAnt2*).

Draft Reports - Laboratory designator, type of report, phase of testing, report date, and version or revision number (e.g, for Laboratory A, draft report, phase IIb, 12 June 06, version 2 would be named *ADRIIb12Jun06v2*).

Final Reports - Laboratory designator, type of report, phase of testing, report date, and version or revision number (e.g, for Laboratory A, final report, phase III, 15 June 06, version 1 would be named *AFRIII15Jun06v1*).

12.18 Font (see also Body Text)

Use Times New Roman font.

12.19 Footnotes

Insert footnotes using the Microsoft Word **Insert, Footnote** command.

Footnote text is 10 pt. type.

The footnote text always appears on the page that contains the footnote mark.

Always indicate a footnote with a superscripted number.

See the **Tables** entry for formatting of footnotes in tables.

12.20 Front Matter (see also individual entries for parts)

All documents that will be sent to the project coordinators and study management team must contain front matter.

Front matter always includes:

Title page

Table of Contents

Front matter may also include (in the following order):

List of Tables

List of Figures

List of Acronyms and Abbreviations

Acknowledgements

Preface

Executive Summary

The preface and executive summary are formatted similarly to body text sections.

Use continuous lowercase Roman numerals for page numbering in all front matter sections.

Page numbers are continuous throughout all front matter sections.

12.21 Headings

Use 12-point type for section headings.

Format headings as follows:

1.0 **HEADING 1** (bold, all caps).

1.1 **Heading 2** (bold, initial caps).

1.1.1 Heading 3 (underlined, initial caps).

1.1.1.1 *Heading 4* (italics, initial caps).

Do not use headings below fourth level.

Set heading numbers flush left.

Indent heading text at 0.7 inches from left margin.

12.22 Headers and Footers (see also Margins, Pagination)

Include headers and footers in every document.

The headers for draft and final reports must include:

The laboratory designation, report type, and phase (e.g., *Laboratory A Draft Report – Phase I*)

The date of document preparation, editing, or other revision (italics) in dd/month/yyyy format (e.g., 17 March 2006).

- On a portrait-oriented page, tab the date to 6.25 inches, right-aligned.
- On a landscape-oriented page, tab the date to 7.75 inches, right-aligned.

In an appendix, include the entire appendix designation in the header.

Use single spacing in the header.

Italicize all text in the header.

See the header of this style guide for an example.

The document footer contains only the page number. The page number is to be centered and in 1 point Times New Roman font.

12.23 Highlighting Text for a Reviewer

When highlighting text for a reviewer, use only the **Highlight** tool (shown below).

Do not use colored shading (i.e., **Format, Borders and Shading**) for highlighting.

12.24 i.e.,

Use the format shown for the abbreviation for *that is to say*.

12.25 Initial Caps

Initial caps means capitalize all words except articles and prepositions. If an article or a proposition is the first word, capitalize it.

12.26 Italics

Use italics for textual elements as indicated in this guide, and for general emphasis.

Specifically use italics for:

Words as words (e.g., The word *basically* is almost always unnecessary and should be avoided in concise writing.)

Publication titles

A word used in an unfamiliar way (e.g., These effects include *pitting* of corneal epithelial cells, *loosening* of epithelium, *roughening* of the corneal surface and *sticking* of the test substance to the cornea.)

To set off single letters from other text (e.g., samples *a* and *b* were tested…)

Foreign words

12.27 Line Numbers

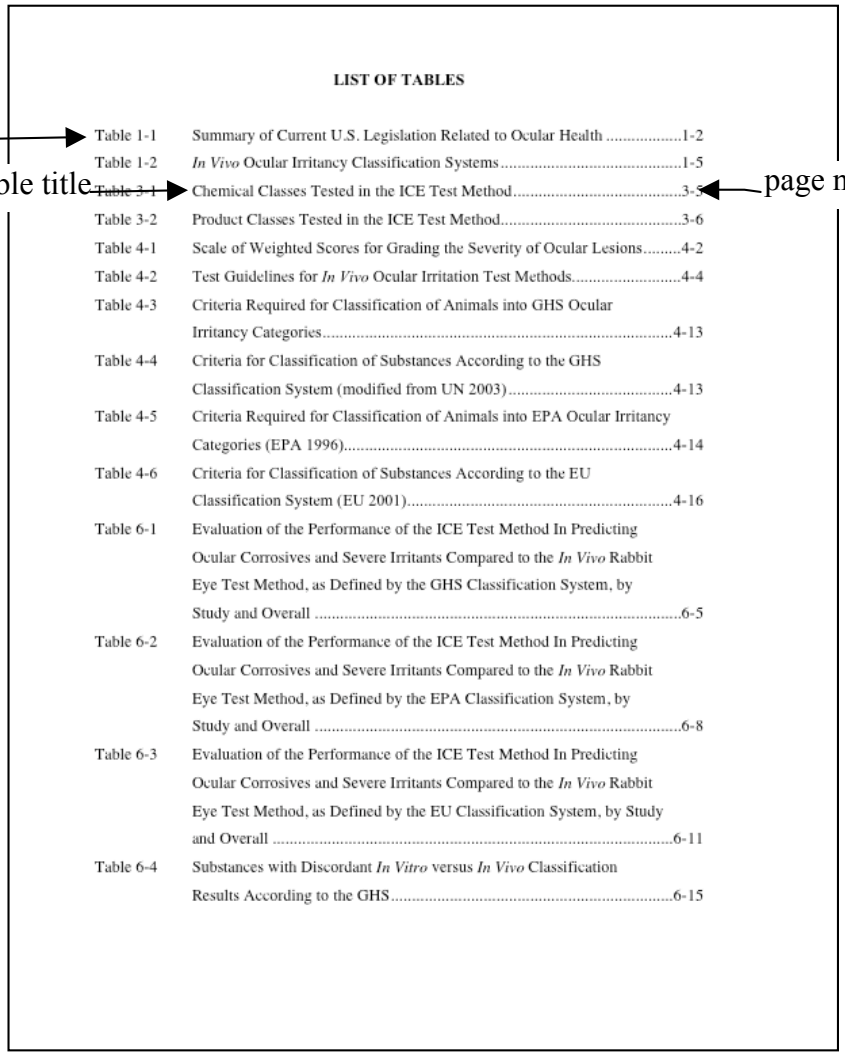
Use line numbers in all reports. Line numbers are continuous throughout the document.

Do not use line numbering on the title page. Begin line numbering in the table of contents.

12.28 Line Spacing

Line spacing is 1.5 lines.

12.29 Format the list of tables and the list of figures similarly. Refer to the following example.



Align the figure or table number flush left.

Indent the figure or table title 1.0 inch.

Set a right-aligned tab at 6.25 inches with a dotted leader for the page number.

12.30 Justification

Use left justification for document text.

12.31 Margins

Set all page margins to 1 inch.

Set the header and footer to 0.5 inches from edge.

12.32 Numbers

When they occur in the text, spell out the numbers zero through nine, unless the number:

Is part of a date (see **Dates**)

Is used as a noun (e.g., Table 1, Sample 2, a value of 8)

Is a quantity followed by a metric unit (e.g., …3 mL were used…)

Appears in a list containing numbers below and above 10 (e.g., 6, 10, and 12 rabbits)

Use numerals for any number greater than nine, unless the number begins a sentence.

For numbers greater than 999, use commas to separate three-digit groups (e.g., 1,000; 546,074; 2,000,000)

Write all decimal numbers as numerals and use a leading zero (e.g., 0.3).

Avoid sentences that begin with awkward constructions (e.g., 5 g of test substance was weighed out …) *Rewrite* so the number does not occur at the beginning of the sentence (e.g., Each assay contained 5 g of test substance…).

12.33 Numbered Lists (see Bulleted and Numbered Lists)

12.34 Page Breaks

Because they often must be deleted and reset at each version, minimize the use of hard page breaks in a draft document.

12.35 Pagination

Include page numbers in all documents. Position in the footer and center justify.

Use 10 pt. type for page numbers.

12.36 Quotation Marks (see Emphasis, Italics)

Use quotation marks only for direct quotations.

Do not use quotation marks for emphasis.

Always place a period or a comma inside quotation marks.

Always place a colon or a semicolon outside quotation marks.

If a question is a direct quotation, place the question mark inside the quotation marks.

If a question is not a direct quotation, place the question mark outside the quotation marks.

Use only one ending punctuation mark in a sentence that contains quotation marks.

If a question occurs both outside and inside of a direct quotation, use only one question mark and place it inside the quotation mark.

If a direct quotation contains a spelling or grammar error or contains factually incorrect information, insert [*sic*] immediately after the questionable material to indicate that the error occurred in the source document. *Sic* is a Latin word meaning *thus* or *so*.

12.37 References Section

Use the format in the EHP *Instructions for Authors*, found at http://www.ehponline.org/docs/admin/edpolicy.html#refe, for references.

Exception: EHP allows the use of et al. after the first six authors; this study will require that all authors be listed.

12.38 Spacing

Separate sentences with one space.

Insert one space after a colon or semicolon.

Always put a space between a number and its unit.

When a symbol precedes a number, do not put a space between the symbol and the number. (e.g. $2.00, quantity <1).

12.39 Section Numbering

Section numbering is in decimal format. See the **Headings** entry for an example.

12.40 Significant Digits

When entering numerical data or the results of calculations into body text or tables, follow the standard rules for significant digits. This may result in tabulated numbers with different numbers of digits after the decimal point.

12.41 Symbols

Use only symbols from the Symbol and normal text fonts.

12.42 Tables

Do not create a table that goes outside of the page margins.

Do not allow a table row to break across a page.

Left-justify all tables, even if the table does not fill the page horizontally.

Preferably, place a table immediately after the paragraph that first cites the table. If this is not possible, place the table as near to this paragraph as possible.

Do not place a table in the middle of a paragraph.

Use 11 point Times New Roman or smaller for table text.

Footnote text following a table is one size smaller than the table text.

For tables that occur in the body of a document, do not use a font size smaller than 8 pt.

Shade the table heading row 10% gray.

Note that column headings and row items may be aligned in table cells as desired, however, maintain consistency of alignment in individual tables or in groups of related tables (i.e., tables that show the same type of data or results.)

Indicate footnotes within a table with superscripted numbers; do not use letters to indicate footnotes. Superscripted symbols (e.g., *, **, #, @) may be used to indicate a footnote if the same symbol is applied repeatedly to different items within a table, or if a numerical footnote would cause confusion (e.g., with an exponent). For example, a * symbol might be used in a table of chemical substances to indicate which substances are insoluble in water.

Definitions of abbreviations used in a table need not be numbered in a footnote. Multiple definitions, listed in alphabetical order and separated by semicolons, may be included in one footnote. (e.g., n=Number; SCNM=Study Criteria Not Met).

Place table footnotes in the following order:

Abbreviations

Footnotes indicated by symbols

Footnotes indicated by numbers

Other than noted above, format table footnotes like text footnotes (see **Footnotes).**

Always right justify CASRNs in a table.

Always left justify Substance Names in a table.

The following table is properly formatted.

Substance Name	CASRN	ER TA Agonist Activity[1]	Anticipated Difficulty
17-α ethinyl estradiol[2]	57-63-6	+++	
Butylbenzyl phthalate	85-68-7	++	
p-n-nonylphenol	104-40-5	++	
o.p-DDT[2]	789-02-6	+	Can potentially "stick" to plastic tissue cultureware
Flavone[2]	525-82-6	+	
Genistein	446-72-0	+	Relatively insoluble
Atrazine[2]	1912-24-9	-	Cytotoxic
Vinclozolin	50471-44-8	-	

Abbreviations: CASRN = Chemical Abstracts Service Registry Number

[1]+++ Indicates that the substance was strongly active (EC_{50} value was <0.001μM); ++ indicates that the substance was moderately active (EC_{50} value was between 0.001 and 0.1μM); + indicates that the substance was weakly active (EC_{50} value was >0.1μM), or a positive response was reported without an EC50 value. The EC_{50} is the effective concentration that causes half-maximal activation of the receptor.

[2]Tested for agonism.

12.43 Table of Contents (see also Front Matter)

Any document may have a table of contents if the authors think it is necessary.

Any document that contains more than one chapter or section must have a table of contents.

An individual chapter or section may have its own table of contents if the authors think it is necessary; however, if this format is used, all chapters or sections must have a table of contents.

If a document is composed of multiple electronic files, Microsoft Word's automated table of contents feature cannot be used. The feature can be used if the document is a single electronic file.

Format the table of contents as in the following example (all text is Times New Roman, 12 pt.):

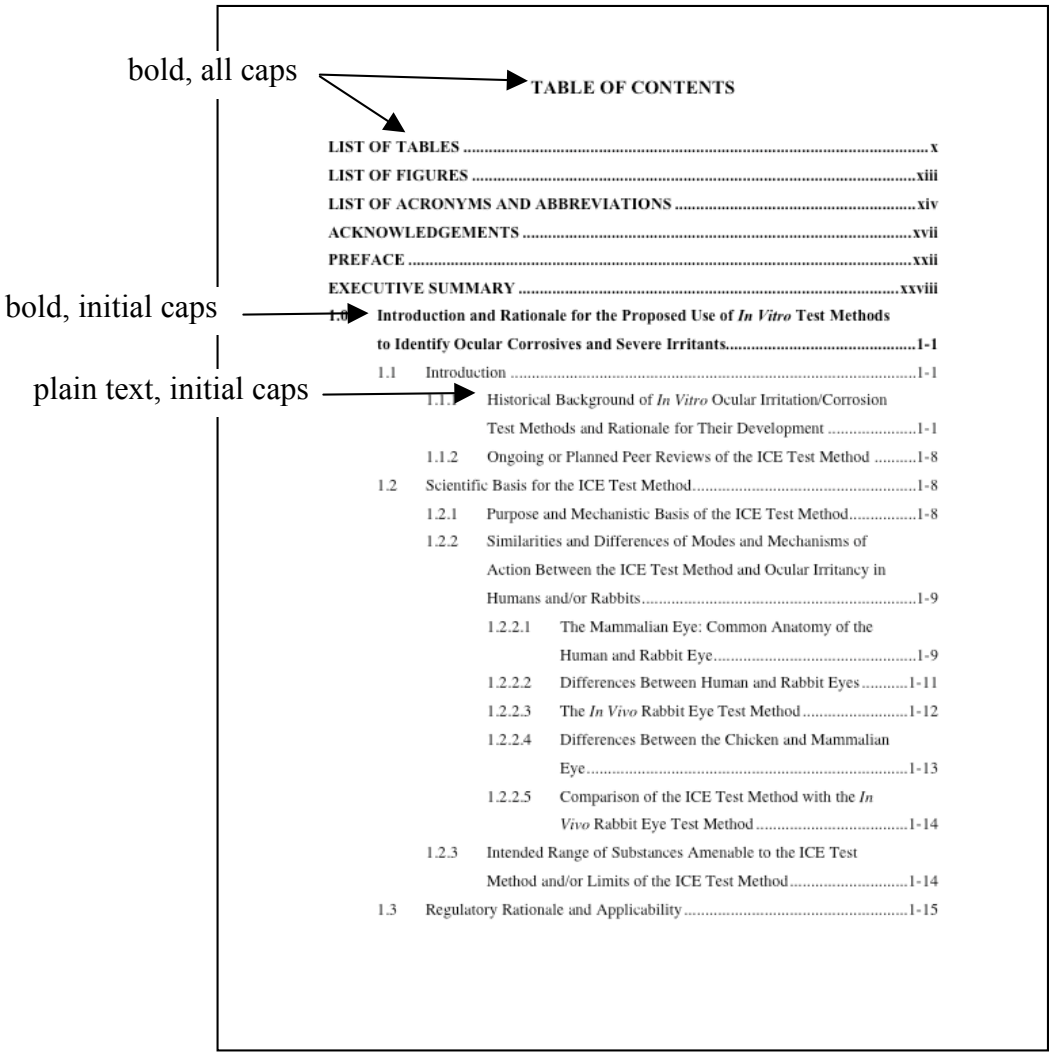


TABLE OF CONTENTS

Align first-level headings flush left. Indent headings for each succeeding level 0.5 inches from the preceding level.

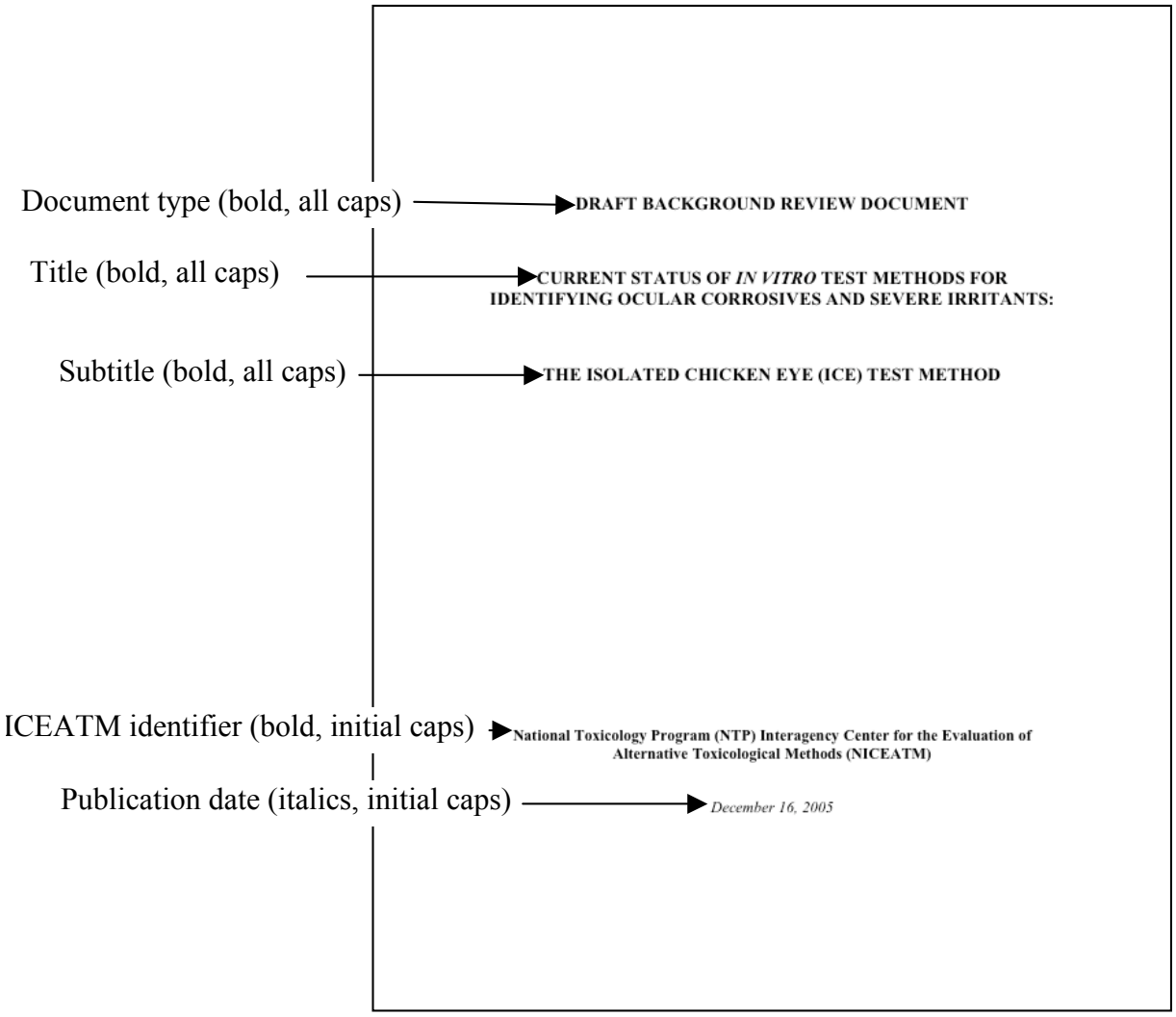
Set a right-aligned tab with a dotted leader at 6.25" for the page number.

12.44 Tabs

Use the TAB key instead of the space bar to set spacing across the page.

12.45 Title Page

Format the title page as in the following example (all text is Times New Roman, 12 pt.):



12.46 Trademark

Do not use a trademark to describe a generic (e.g., use the word *photocopy*, not *Xerox®)*.

When using a trademark in the text, ensure that the proper symbol (i.e., ™ or ®) is used with the trademark. If unsure about which symbol is correct, verify with the owner of the trademark, either online or by contacting the company.